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## Increased Numbers of GABAergic Neurons Occur in the Inferior Colliculus of an Audiogenic Model of Genetic Epilepsy

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The numbers of GABAergic neurons as determined by GAD immunocytochemistry and total neurons as determined from Nissl preparations were counted and classified at the light microscopic level in the inferior colliculus (IC) of the genetically epilepsy-prone rat (GEPR) and the non-epileptic Sprague–Dawley (SD) strain of rat. GAD-positive neurons are abundant in the IC in all 3 subdivisions. Several sizes of multipolar neurons as well as medium-sized bipolar or fusiform neurons are GAD-positive. GAD-positive punctate structures that were interpreted to be axon terminals and transversely-sectioned dendrites and preterminal axons are abundant in the IC of both the GEPR and SD. A dramatic increase in the number of GAD-positive neurons occurs in the GEPR as compared to the SD. This increase is most evident in the middle of the rostrocaudal extent of the IC. Although the increase is statistically significant in all subdivisions of the IC, it is most pronounced in the central nucleus, especially the ventral lateral portion. Within the central nucleus, the increase in the number of GAD-positive neurons is due to a selective increase in the small (200%) and medium (90%) cell body size populations (10–15  $\mu\text{m}$  and 15–25  $\mu\text{m}$  in diameter, respectively). Concomitant with this increase in the number of GAD-positive neurons, an increase in total numbers of neurons occurs as determined from Nissl preparations. A 100% increase in the number of small neurons and a 30% increase in the number of medium-sized neurons occur in the GEPR as compared to the SD rat. The proportion of GAD-positive neurons to total neurons is also increased in the GEPR. Approximately 25% of the neurons in the IC in SD rat are GAD-positive, while about 35% of the neurons in the GEPR are GAD-positive. These data demonstrate an anatomical difference in the IC of the GEPR as compared to the SD which appears to be preferential for the GABAergic system.

### INTRODUCTION

The genetically epilepsy-prone rat (GEPR) exhibits severe generalized motor seizures in response to intense auditory stimuli. Lesion studies indicate that the primary neuronal pathways involved in the manifestation of audiogenic seizures are subcortical because ablation of auditory cortex does not prevent seizures<sup>1,2,13,14</sup>. Lesions of the inferior colliculus (IC) prevent seizures and other data support the notion that the IC is involved with audiogenic seizures. Pharmacological studies indicate that GABA and benzodiazepine binding is abnormal in the IC<sup>5</sup>. In addition, infusion of GABA agonists into the IC attenuates seizures and conversely, infusion of GABA antagonists causes seizures<sup>2,3</sup>. Also, an increase in after discharge-like responses similar to that observed in

other types of seizures has been observed in the IC of the GEPR<sup>4</sup>. These studies indicate several abnormalities within the IC of the GEPR, including a possible loss of GABA-mediated inhibition.

A number of studies have indicated that a decrease in the neurotransmitter, GABA, can cause a significant loss of CNS inhibition and result in a hyperexcitable state<sup>18,25</sup>. Anatomical studies of focal models of epilepsy by Ribak and coworkers are consistent with this notion because they have demonstrated a preferential loss of GABAergic terminals at epileptic foci created by alumina gel in monkeys<sup>28–30</sup> and in the isolated cortical slab model of focal epilepsy<sup>31</sup>. Only one genetic model of epilepsy, the seizure sensitive gerbil, has been analyzed using GAD immunocytochemistry and the results are the opposite to those observed in the focal models<sup>24</sup>.

We wanted to know if changes in the GABAergic system occurred in another genetic model of epilepsy. To determine if the GABAergic system was different in the IC of the GEPR as compared to the non-seizuring Sprague–Dawley rats, we utilized immunocytochemical methods to localize GABAergic neurons. In addition, total cell counts as determined with an analysis of Nissl preparations were performed. Together, these results indicate that the GABAergic system in this genetic model of epilepsy is different from that found in the normal rat. A preliminary report of this work was presented at the 14th Annual Meeting of the Society for Neuroscience<sup>35</sup>.

## MATERIALS AND METHODS

### Rats

The genetically epilepsy-prone rats (GEPRs) used in this study were offspring of the UAZ strain received from Dr. Phillip Jobe, LSU School of Medicine, Shreveport, LA. The non-seizuring Sprague–

Dawley (SD) rats were obtained from Simonsen Laboratories, Gilroy, CA. Both groups of animals were tested in a padded box in an acoustic chamber containing two standard electric doorbells (Fig. 1). Both groups were given an audiogenic response score according to the scale established by Jobe et al.<sup>12</sup>. GEPRs were used after receiving three consecutive scores of 9 and SD rats were used if their audiogenic response score was 0.

### Immunocytochemistry

Adult animals were deeply anesthetized with nembutal and injected with 10  $\mu$ l of a 1% colchicine solution into the lateral ventricle to enhance the staining of somata<sup>32</sup>. Twenty-four hours later the animals were transcardially perfused with physiological saline followed by a 4% paraformaldehyde solution. Brains were soaked in a 30% sucrose solution overnight and sectioned in the coronal plane on a sliding freezing microtome the following day. Immunocytochemical localization of glutamic acid decarboxylase



Fig. 1. A GEPR rat shown in tonic extension phase of seizure in padded testing chamber. These rats exhibit a fit of wild running followed by clonus and full tonic extension in response to intense auditory stimuli, in this case two electric doorbells (arrows).

(GAD), the synthesizing enzyme for GABA, was used to identify GABAergic neurons and axon terminals. Every fifth 40  $\mu\text{m}$  section was processed for GAD immunocytochemistry using an anti-GAD serum<sup>22</sup> and an avidin biotin peroxidase complex (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA). Omission of the primary antiserum served as the immunocytochemical control. The ABC method employs a biotinylated antibody and an avidin biotinylated horseradish peroxidase complex. The advantage of this ABC method is that the sensitivity is superior to other immunoperoxidase staining techniques. Briefly, this method uses solutions that are dissolved in Tris buffered saline (TBS). 'Wash' refers to 3 10-min rinses in TBS. First the sections are incubated in 0.1 M D.L-lysine in 10% normal rabbit serum for 12 h and then incubated in the primary antiserum raised in sheep against GAD for 24–48 h. Following a wash the sections are incubated in the biotinylated secondary antibody, rabbit antisheep IgG (1:227) for 45 min. After another wash the sections are then incubated for 45 min in a mixture of avidin and biotinylated peroxidase complex (1:114). Following a wash the sections are reincubated with the secondary antibody for 30 min, washed and reincubated in the avidin biotinylated peroxidase complex for 30 min. The reason for this reincubation is to form a double bridge which results in enhanced immunostaining<sup>23</sup>. After a wash the sections are reacted for 15–30 min in a DAB solution (6 mg diaminobenzidine/10 ml TBS + 0.002% hydrogen peroxide). Following a wash the sections are mounted on gelatin subbed slides, air dried, and then reacted in 0.005%  $\text{OsO}_4$  for 60 min. Finally, the slides are defatted, dehydrated and coverslipped. The criteria for identifying GAD-positive neurons included distinct cytoplasmic boundaries and the presence of dark brown reaction product within the perikaryal cytoplasm. All GAD-positive cell bodies in 4–8 sections 200  $\mu\text{m}$  apart throughout the rostrocaudal extent of the IC were counted. Somata from a representative 62,500  $\mu\text{m}^2$  grid in the ventral lateral portion of the central nucleus of the IC (ICCN) were counted and classified according to size and morphology. Somata from a representative 31,250  $\mu\text{m}^2$  area were also counted for the pericentral nucleus (PCN) and the external nucleus (EN). Data were analyzed statistically using a Student's *t*-test. To determine if the increase in num-

ber of GAD-positive neurons was present in other brain regions, the oculomotor nucleus (cnIII) and the medial superior olive (MSO) were analyzed in 5 sections 200  $\mu\text{m}$  apart in two SD rats and 3 GEPRs.

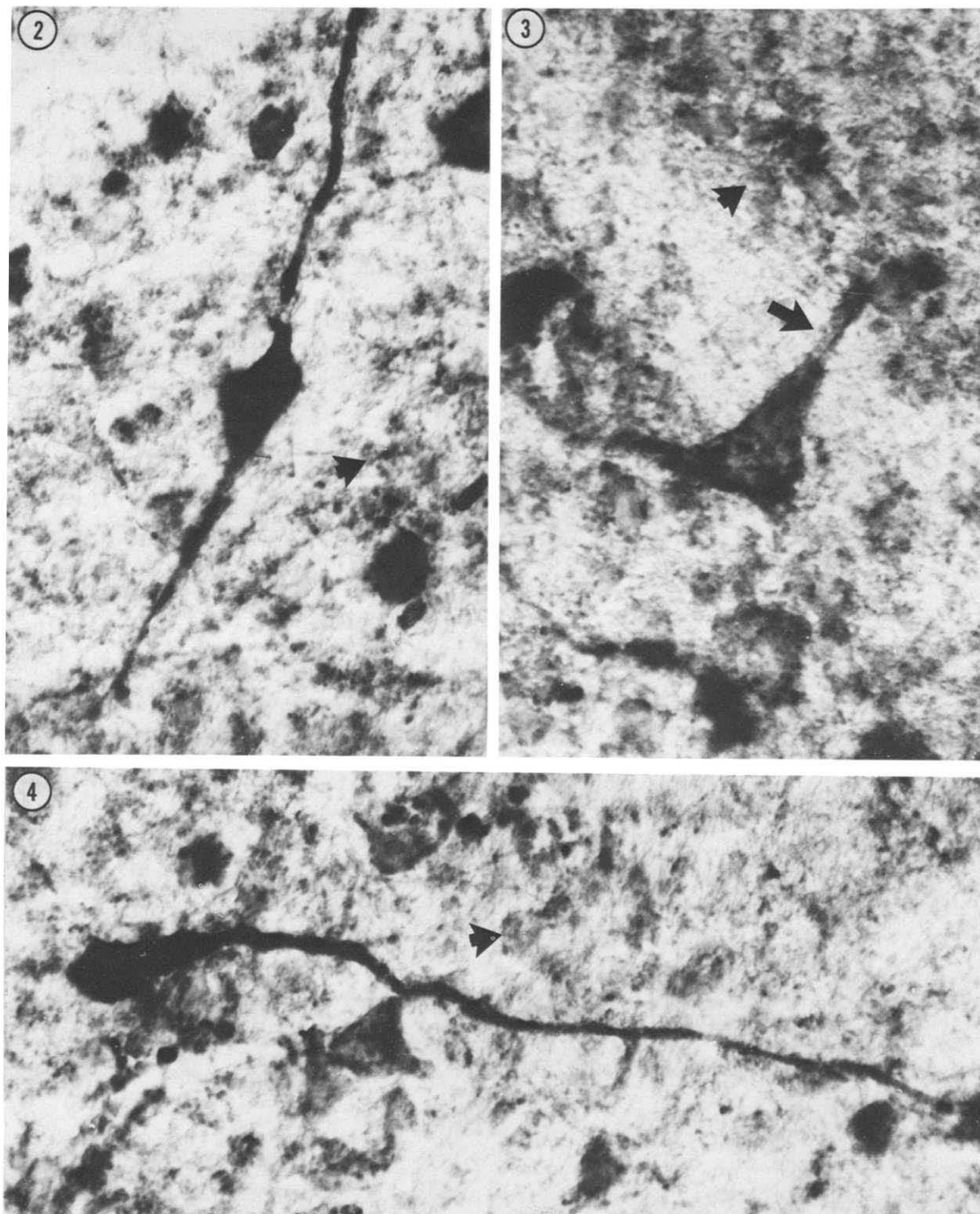
#### *Nissl preparations*

Two animals from each strain were perfused with 4% paraformaldehyde and their brains were embedded in paraffin. The brains were sectioned in the coronal plane on a rotary microtome at a thickness of 10  $\mu\text{m}$ , mounted on slides and stained with cresyl violet. Every 10th section throughout the rostrocaudal extent of the IC was analyzed. Cell bodies were drawn from a representative 62,500  $\mu\text{m}^2$  grid in the ventral lateral portion of the ICCN using a camera lucida. Somata were classified as small (10–15  $\mu\text{m}$  in diameter), medium (15–25  $\mu\text{m}$  in diameter) or large (greater than 25  $\mu\text{m}$  in diameter), and the average cell number in each size group was tabulated. Data were statistically analyzed using a Student's *t*-test. Cell bodies were also counted from every 10th section in cnIII and MSO. Six to 10 sections from each of these nuclei were analyzed. Since both cnIII and MSO are small, a large sample of the total number of neurons in each nucleus could be counted in these sections.

## RESULTS

#### *Distribution of GAD-positive structures in the IC of the Sprague-Dawley rat*

Numerous GAD-positive neuronal somata were found in the IC in all 3 of its subdivisions, the central nucleus (ICCN), the pericentral nucleus (PCN) and the external nucleus (EN). The ICCN has a heterogeneous population of somata displaying several sizes and morphological characteristics. In addition, dendritic staining of some GAD-positive somata demonstrated that some cells had dendrites oriented within a lamina (Fig. 4), or across a lamina (Fig. 2) or both (Fig. 3). Multipolar neurons of all sizes (10–30  $\mu\text{m}$  in diameter) were found in the ICCN (Figs. 5 and 6). In addition, some somata were bipolar or fusiform (Fig. 7). However, the majority of the GAD-positive somata were small multipolar type neurons (Figs. 6 and 8). The morphology of these multipolar cells whose dendrites radiate in all directions within and/or across several laminae suggests that they are



Figs. 2–4. GABAergic neurons in the inferior colliculus identified by immunocytochemical localization of GAD. Fig. 2. Bipolar GAD-positive neuron with dendrites oriented perpendicular to the laminae in the ventral-lateral portion of the central nucleus. Fig. 3. Pyramidal-shaped GAD-positive neuron with dendrites at right angles to one another. One crosses a lamina (arrow) and the other courses within another lamina. Fig. 4. Fusiform, GAD-positive neuron with a dendrite oriented within a lamina. Note the abundance of dark, punctate structures (arrowheads) that represent GAD positive axon terminals or transversely-sectioned dendrites. 900 $\times$ .

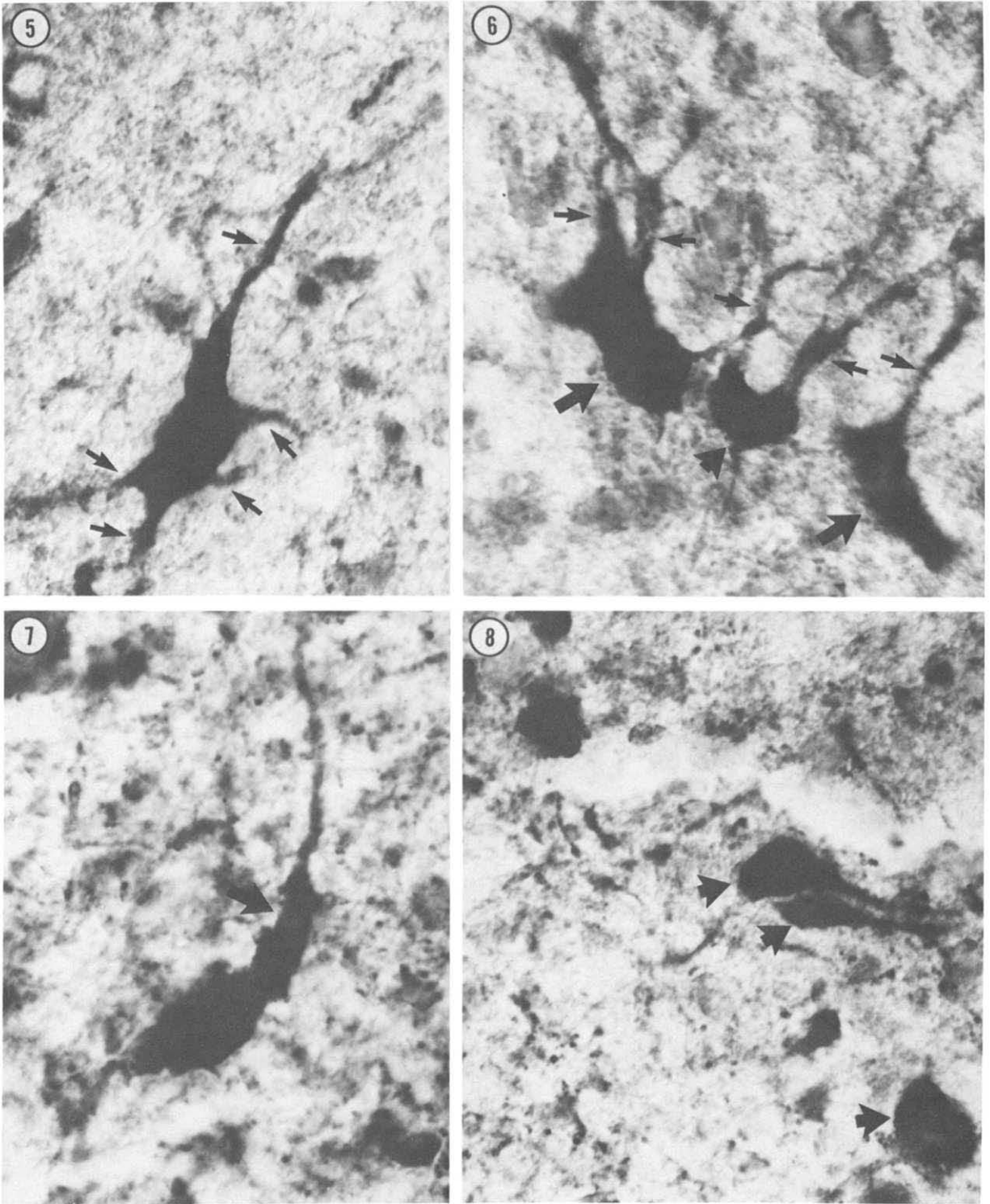


Fig. 5. Large, multipolar GAD-positive neuron with dendrites (arrows) oriented in several directions. 900 $\times$ .

Fig. 6. Two large (large arrows) and one small (arrowhead) multipolar GAD-positive neurons with dendrites (small arrows) oriented in the same direction. 900 $\times$ .

Fig. 7. Large fusiform neuron with bulbous stem dendrite (arrow). 900 $\times$ .

Fig. 8. Several small neurons are shown (arrowheads). These small cells and the one shown in Fig. 6 are the type that are increased the most in number in the GEPR. 900 $\times$ .



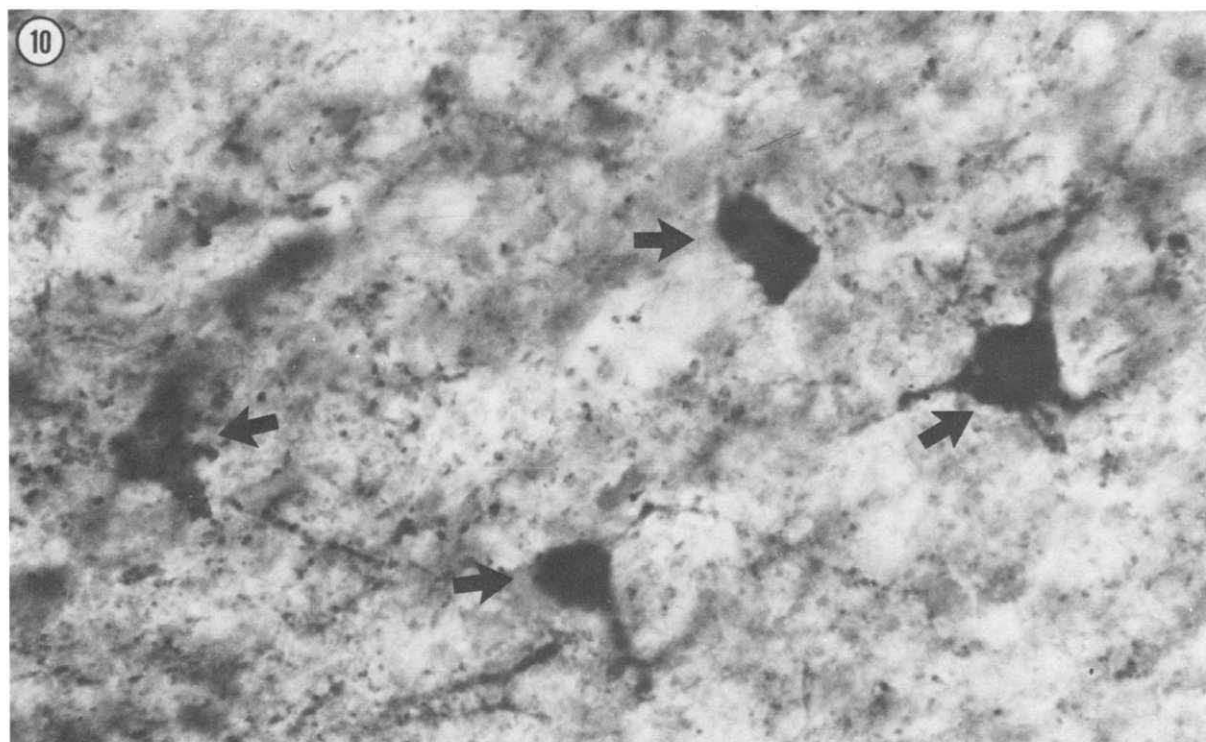
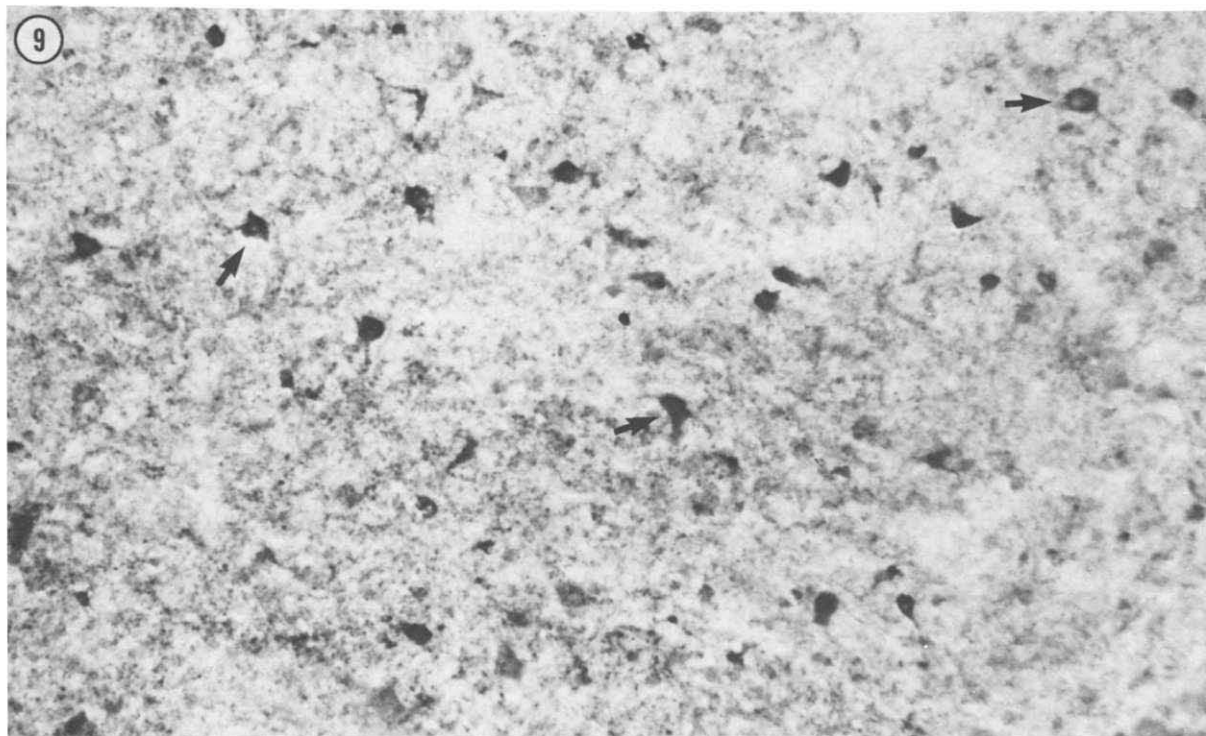


Fig. 9. Photomicrograph of the ventral lateral portion of the central nucleus of the inferior colliculus of the Sprague-Dawley rat. The typical distribution of GAD-positive somata (arrows) is shown. 225  $\times$ .

Fig. 10. An enlargement of another field from a region similar to Fig. 9. Four GAD-positive somata are shown (arrows). 640  $\times$ .

local circuit neurons. In contrast, bipolar or fusiform cells with dendritic staining had polarized dendrites oriented either across or within a lamina (Figs. 2 and 4). Based on the shape and dendritic orientation of these latter cells, they appeared similar to previously described projection neurons<sup>36</sup>. GAD-positive neurons in the PCN were small, oval or fusiform, and some were multipolar. The EN contained medium-sized spindle or triangular-shaped GAD-positive cells, some of which were multipolar.

The IC also contained GAD-positive punctate structures that represent GAD-positive axon terminals and perhaps some small transversely-sectioned dendrites and preterminal axons (Figs. 2–4). GAD-positive puncta were more densely concentrated in the ICCN than in the PCN or the EN. The puncta appeared mainly in the neuropil but some were occasionally observed around unstained somata. The staining of GAD-positive somata was usually too dark to discern if GAD-positive puncta were found adjacent to them. However, GAD-positive puncta were found in close association with some paler stained large GAD-positive somata.

#### *Distribution of GAD-positive structures in the IC of the GEPR*

The GAD-positive neuronal somata in the IC of the GEPR were similar to those found in the SD rat. For example, GAD-positive somata were present in the 3 subdivisions of the IC and displayed a wide variety of sizes and shapes similar to that found in SD rats. Dendrites of multipolar cells were observed to radiate in all directions whereas dendrites of fusiform cells either crossed or stayed within a lamina. In addition, the majority of GAD-positive cells were small and multipolar.

One major difference was that many more GAD-positive cells occurred in the GEPR than in the SD rat. This difference was apparent at low magnifications of the ventral lateral portion of the ICCN (Figs. 9 and 11). Enlargements of representative regions of the ICCN (Figs. 10 and 12) revealed that the distribution of large cells in the SD rat and the GEPR was similar, but that the small- and medium-sized GAD-positive neurons were dramatically increased in number in the GEPR. Note that in the field shown in Fig. 10 only 4 GAD-positive neurons are shown in the SD preparation. In contrast, at least 10 GAD-positive

somata are present in a similar region in the GEPR (Fig. 12).

GAD-positive puncta representing axon terminals and some transversely-sectioned axons and dendrites were abundant in all 3 subdivisions of the IC of the GEPR. The apparent density of the puncta was homogeneous throughout the IC. Since the 40- $\mu$ m thick sections had such dense staining in both groups of animals, counting puncta in these preparations was not possible.

#### *Quantitative analysis*

A quantitative analysis of GAD immunocytochemical preparations confirmed the observations noted in our qualitative descriptions (see above). A significant increase ( $P < 0.001$ ) in the number of GAD-positive neurons occurred in the IC of the GEPR as compared to the SD rat. GAD-positive neurons were counted from representative grids in each region of the IC: EN, PCN, ventral lateral ICCN and dorso-medial ICCN (Fig. 15). Although there were more GAD-positive cells present in the GEPR in all subdivisions of the IC, the most dramatic increase was in the ICCN, especially the ventral-lateral portion. Throughout the rostrocaudal extent there were more GAD-positive neurons in the GEPR than in the SD (Fig. 14). This increase was most prominent in the center of the rostrocaudal extent where there was a 200% increase in the number of small cells (10–15  $\mu$ m in diameter) and a 90% increase in the number of medium-sized cells (15–25  $\mu$ m in diameter) in the GEPR as compared to the SD rat (Fig. 16). In contrast, the number of GAD-positive large cells (diameter  $> 25 \mu$ m) was similar in both groups of animals. A representative example of the number and location of GAD-positive somata in the IC of both types of rats as well as the boundaries of the subdivisions of the IC are shown in Fig. 13.

The Nissl preparations revealed a heterogeneous population of small, medium and large neurons in the ICCN. Consistent with the quantitative data on GAD-positive cells, there were more neurons in the GEPR than in the SD rat (Figs. 17 and 18). This increase occurred in the numbers of small- (100%) and medium-sized (30%) neurons, consistent with the increase in the GAD-positive cell types (Fig. 19). No regional differences were observed throughout the rostrocaudal extent of the ICCN, so the mean num-



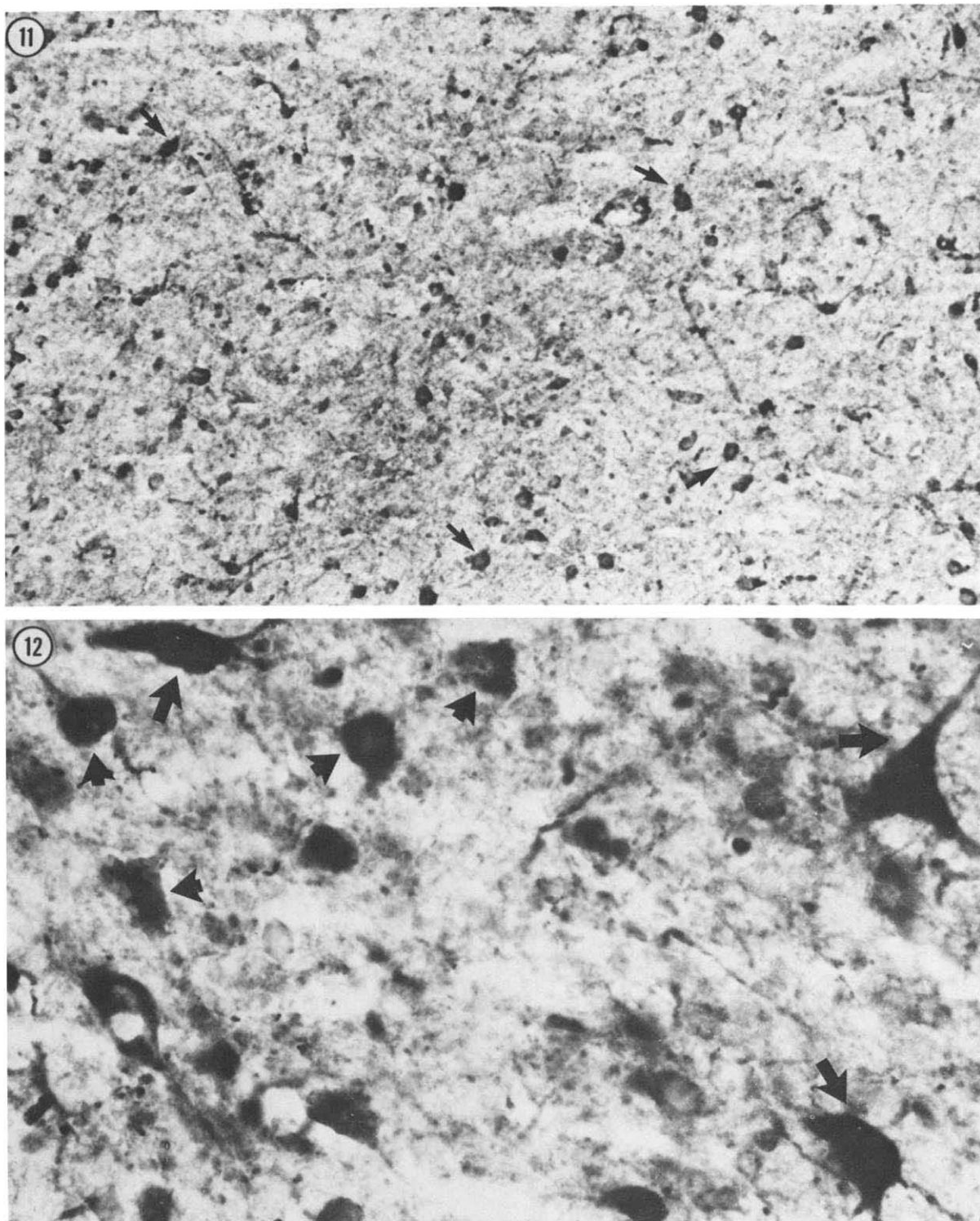


Fig. 11. Photomicrograph of the ventral-lateral portion of the central nucleus of the inferior colliculus of the GEPR at an equivalent level to that shown for the SD rat. The typical distribution of GAD-positive somata (arrows) is shown. At this magnification, it is apparent that there is an increase in the number of GAD-positive neurons. 225 $\times$ .

Fig. 12. An enlargement of another representative field in the same region of the GEPR of the IC as Fig. 10. The relative number of large neurons (arrows) is similar to that observed in the SD (cf. Fig. 10). However the number of small cells (arrowheads) is increased. 640 $\times$ .

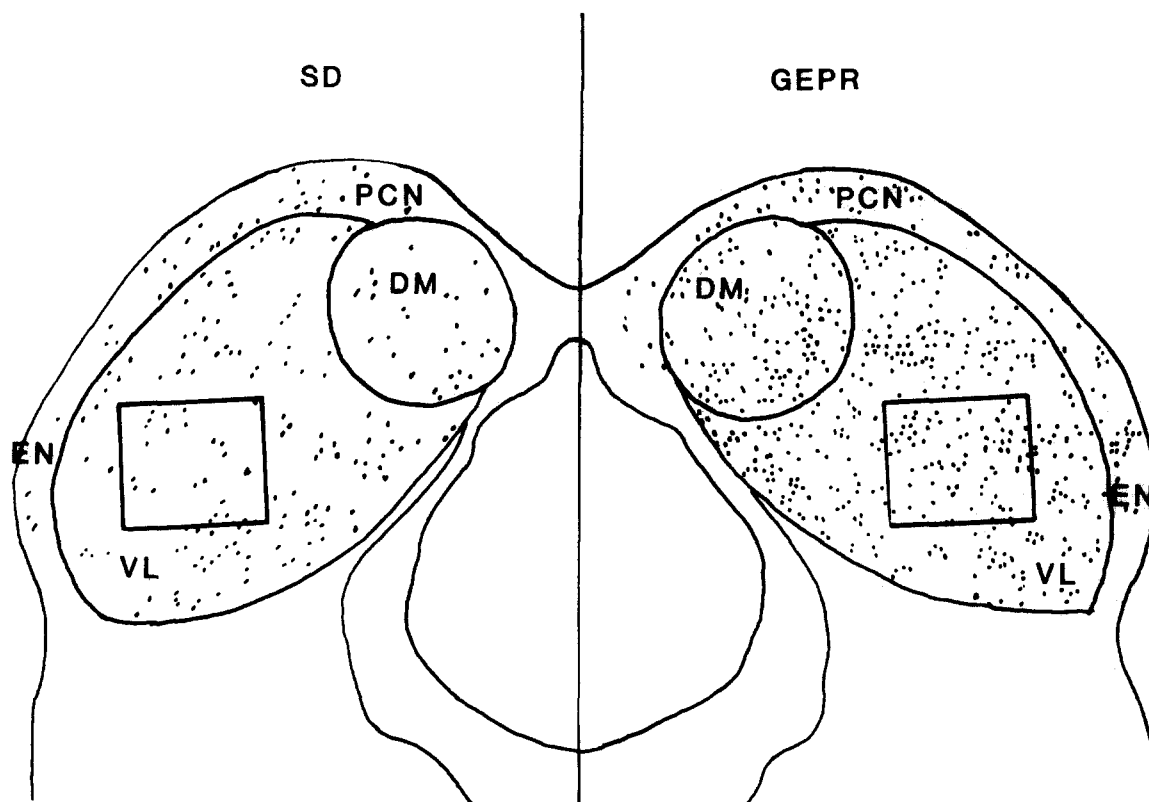


Fig. 13. Line drawing of a transverse section through the middle portion of the rostrocaudal extent of the IC. Lines represent boundaries of the subdivisions within the IC (EN, PCN, DM, VL — see text for description). Dots represent GAD-positive somata counted from one representative section in the GEPR and the SD, respectively. Boxes represent the region from which Figs. 9–12 were photographed and representative grids for quantitative analysis (Figs. 15, 16 and 19) were located.

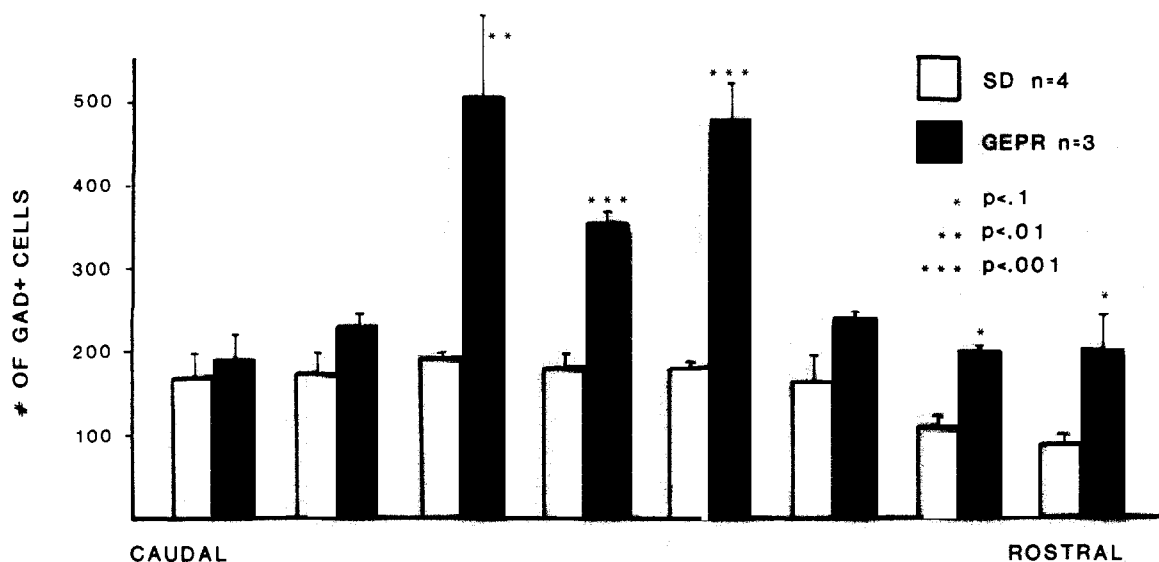


Fig. 14. Histogram showing the total numbers of GAD-positive cells counted from 8 similar levels of the rostrocaudal extent of the IC from both GEPR and SD rats. The GAD-positive neurons are more numerous throughout the IC of the GEPR than in the SD. The increase is most dramatic in the middle of the rostrocaudal extent. In animals where 8 sections were not available, the sections were matched to the appropriate levels.

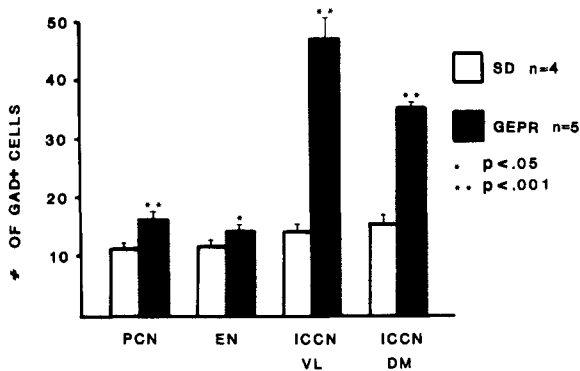


Fig. 15. Histogram showing the number of GAD-positive cells counted from representative  $31,250 \mu\text{m}^2$  areas in the PCN and the EN and from  $62,500 \mu\text{m}^2$  areas in the ventral-lateral (VL) and the dorsomedial (DM) portion of the ICCN. Although the increase in the numbers of GAD-positive neurons is statistically significant for all regions of the IC in the GEPR as compared to the SD, the increase is most dramatic for both portions of the ICCN.  $n$  = number of rats; 20–25 areas were sampled in the GEPR and 25–43 areas were sampled in the SD to obtain the averages.

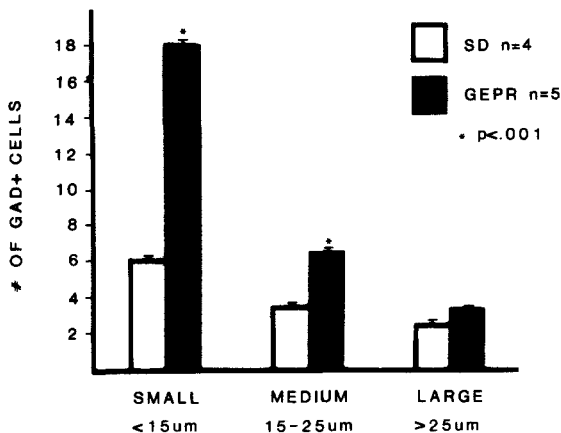


Fig. 16. Histogram showing the average number of small, medium and large cells, based on diameter of soma, counted from  $62,500 \mu\text{m}^2$  areas in the ICCN, ventral-lateral portion. The GEPR displays a 200% increase in the number of small GAD-positive cells ( $P < 0.001$ ) and a 90% increase in the number of medium sized cells ( $P < 0.001$ ). In contrast, the number of large GAD-positive cells in the GEPR was similar to that for the SD.  $n$  = number of rats; 35 grids from SD brains were sampled and 20 grids from GEPR brains were sampled.

ber represents the averages from all levels of the IC.

To determine if the increased number of neurons present in the IC was found in other brain regions, two other nuclei were chosen for cell quantification analysis in Nissl preparations: the medial superior

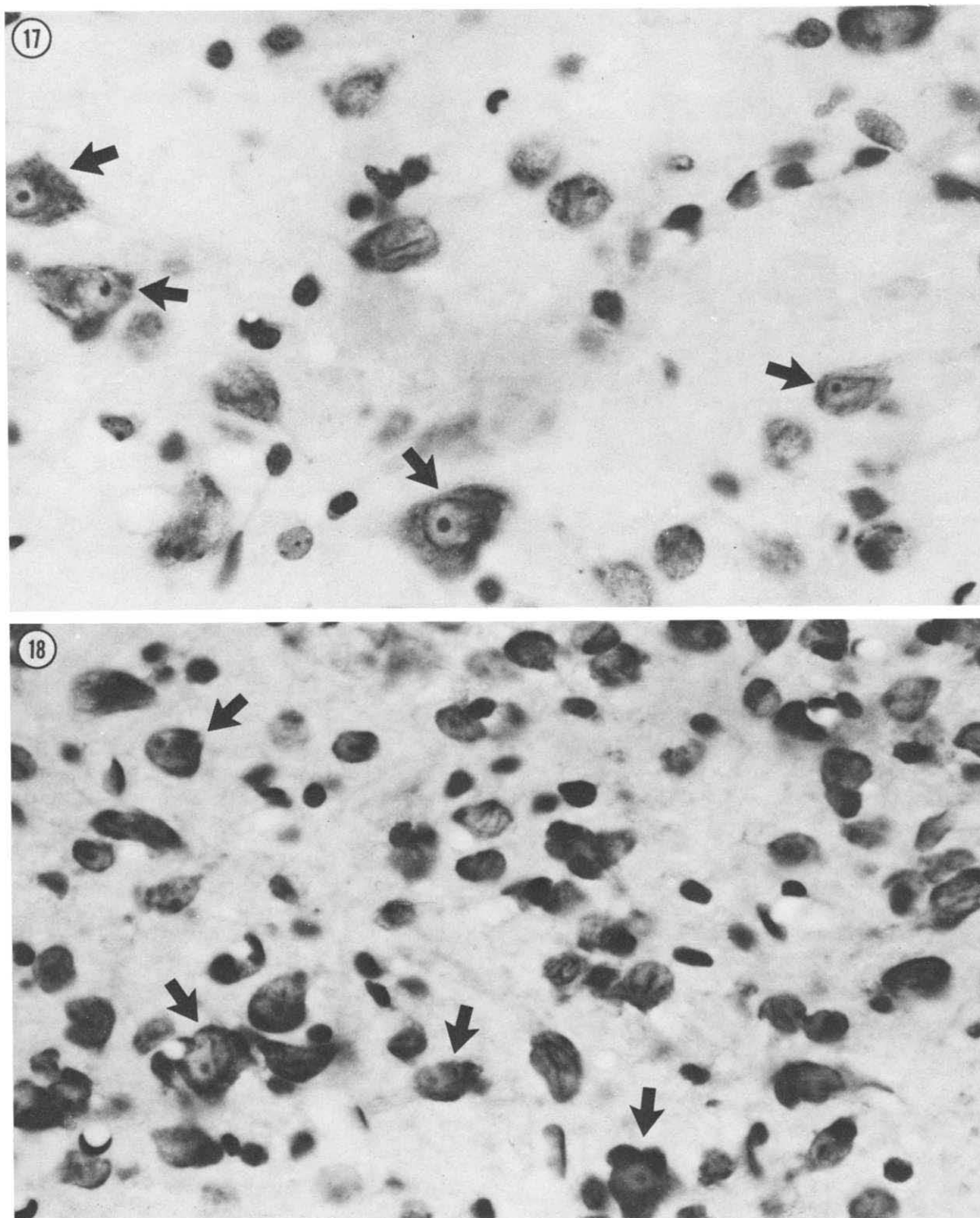
olive (MSO) and the oculomotor nucleus (cnIII). These nuclei were chosen because cnIII is another midbrain nucleus not related to the primary auditory pathway and MSO is another primary auditory brain-stem nucleus. In addition, they are small and have discrete boundaries. Neurons in MSO and cnIII were counted in 6–10 transverse sections  $100 \mu\text{m}$  apart. The results for cnIII were: SD rat,  $n = 2$ , 22 sections measured,  $\bar{x} = 15.86 \pm 0.66$  cells/nucleus/section; GEPR,  $n = 2$ , 19 sections measured,  $\bar{x} = 15.37 \pm 0.57$  cells/nucleus/section. No significant difference was found between the SD rats and the GEPRs using a Student's  $t$ -test ( $t = 0.55$ ,  $df = 39$ ). The results for MSO were: SD,  $n = 2$ , 31 data points taken,  $\bar{x} = 15.4 \pm 0.7$ ; GEPR,  $n = 1$ , 12 data points taken,  $\bar{x} = 12.7 \pm 0.6$ ,  $P < 0.05$ . There was no increase in cell number in the GEPR in these two regions, rather a slight decrease was found in the number of cells in MSO. The number of GAD-positive somata were also counted in these two regions in the immunocytochemical preparations. The results showed no differences between the GEPRs and the SD rats in both brain regions and are consistent with the Nissl data.

## DISCUSSION

### *GAD-positive neurons in the IC*

The ICCN is organized in a laminar arrangement with fascicles of afferent axons interlaced between dendrites and cell bodies. Several sizes of multipolar neurons exist within the ICCN, some of which are probably local circuit neurons<sup>20,26,36</sup>. Some multipolar neurons in all size ranges are GABAergic suggesting that at least some of the local neuronal circuitry is mediated by GABA. Fusiform neurons with both the long axis of their somata and dendrites oriented within laminae have been described previously as principal cells<sup>21,26,36</sup>. Another fusiform principal cell type that has the long axis of its soma and dendrites oriented across several laminae is present within the ICCN<sup>36</sup>. Some of these two types of cells are GAD-positive and these findings suggest that some projection neurons are GABAergic.

Some multipolar neurons as well as one type of principal cell have dendrites that cross several laminae. Since the IC is tonotopically organized<sup>37</sup> and this is reflected by the anatomical lamination, the neurons whose dendrites are oriented across laminae are



Figs. 17 and 18. Nissl preparations obtained from the ICCN of the SD (Fig. 17) and the GEPR (Fig. 18) to show the distribution of neuronal somata (arrows). The GEPR displays a dramatic increase in the number of neuronal somata as compared to the SD. 900 $\times$ .

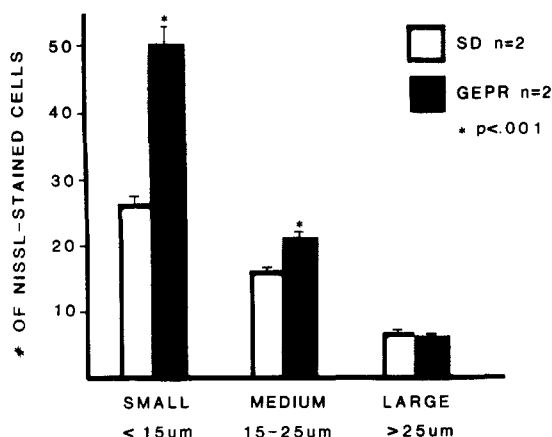


Fig. 19. Histogram showing the average number of small, medium and large Nissl-stained neurons counted from 62,500  $\mu\text{m}^2$  areas in the ICCN, ventral lateral portion. The GEPR displays a 100% increase in the number of small cells and a 30% increase in the number of medium-sized cells in comparison to the SD rats. There is no difference in the number of large neurons in both groups of rats. n = number of animals; 17–24 samples were counted from each animal.

apt to receive input from a group of cells conveying information about several frequency ranges. In contrast, the cells with dendrites oriented within a lamina are most likely receiving information about a more restrictive frequency range. The ICCN is involved in the localization of sound and the inhibitory interactions that are involved in the processing of interaural time and intensity differences could be mediated in part within the IC by intrinsic, small, multipolar cells, many of which are GABAergic.

In Golgi preparations<sup>36</sup> some of the principal cells give rise to axons that enter the brachium of the IC en route to the medial geniculate body (MGB). Since some of these cell types are GABAergic, it is possible that one source of GABAergic terminals in the MGB may arise from these neurons, in addition to those derived from local circuit neurons<sup>33</sup>. The IC also projects to the dorsal cochlear nucleus<sup>27</sup> and various periolivary subnuclei, all of which contain fairly dense concentrations of GAD-positive axon terminals<sup>19,34,38</sup>. The cell type giving rise to this descending auditory pathway to these two regions has not yet been identified. However, since some of each cell type within the IC contains GAD, it is reasonable to expect that at least some of the GAD-positive axon terminals arise from GAD-positive cells in the IC.

Therefore, GABAergic pathways emerging from the IC possibly modulate ascending input.

#### *Increased numbers of GABAergic neurons in the IC of the GEPR*

The GABA hypothesis of epilepsy states that decreases in the neurotransmitter, GABA, can cause a significant loss of CNS inhibition and result in a hyperexcitable state. This hypothesis has been demonstrated in anatomical preparations for certain models of focal epilepsy where a preferential loss of GABAergic terminals and somata are associated with the cortical focus caused by alumina gel in monkeys<sup>28–30</sup>. GABAergic terminals are also selectively lost in the isolated cortical slab model of epilepsy<sup>31</sup>. In both these cases the epileptic activity has been attributed to these deficits in GABAergic transmission. In contrast to these studies, a statistically significant increase in the number of GABAergic neurons occurs in the IC of GEPRs as compared to the SD rats. These results are opposite to those expected from the models of focal epilepsy.

Quantitative analysis of immunocytochemical preparations is difficult because of the inherent capriciousness of the technique. We have tried to circumvent this problem by quantifying only the best stained preparations in both groups. The magnitude of the difference we have observed in these preparations was consistent and warrants the use of these preparations for quantitation. In addition, we never observed as many GAD-positive neurons in the SD rats as found in the GEPRs. Furthermore, the increase in GAD-positive neurons seen in the IC of the GEPR does not seem to be a whole brain phenomena because the numbers of GAD-positive neurons in cnIII and MSO were similar between the two groups. The data for the Nissl-stained preparations are consistent with these findings.

How can this increase in GABAergic cells in the GEPR be responsible for epileptic activity? If the increase in GABAergic neurons results in the increased inhibition of other GABAergic cells which are involved in a tonic inhibition of excitatory projection neurons, then the result would be a disinhibition of projection neurons. Therefore, these data suggest that two different neuronal circuits, loss of inhibition and disinhibition, may cause epilepsy.

The neuronal circuitry in genetic models of epilep-

sy appears to be quite different from the focal models. Data from another genetic model of epilepsy, the seizure sensitive gerbil<sup>24</sup>, supports this notion. In this model increased numbers of GABAergic basket cells occur in the dentate gyrus of the hippocampus of the seizure-sensitive gerbil. Basket cells provide recurrent inhibition to the granule cells, which are excitatory projection neurons. It is possible that the extra GABAergic cells interfere with the normal recurrent inhibition by inhibiting the normal basket cells. Therefore, the disinhibition hypothesis of epileptic activity may also be true for this genetic model.

#### *A specificity in the increase in number of cells*

A 100% increase in the number of small cells and a 30% increase in the number of medium-sized cells is found in the Nissl preparations of ICCN of the GEPR. Thus, the 200% increase in small GABAergic cells and the 90% increase in medium-sized GABAergic cells could, in part, be accounted for by the increase in the total number of cells. The increased numbers of all cells, and GABAergic cells in particular, is the first evidence of an anatomical difference in the IC of the GEPR as compared to the SD. The increase in total cell number along with the increase in GABAergic cell number is interesting because only the small- and medium-sized cells are increased in number, and these are the sizes of GABAergic cells that are increased in number.

The proportion of GAD-positive neurons to total neurons is increased in the GEPR despite the parallel increase in both measurements. For example, about 25% of the total number of small neurons in the SD rat are GAD-positive, whereas about 36% of the total number of small neurons are GAD-positive in the GEPR. A similar increase occurs in the proportion of GAD-positive medium-sized neurons, with about 25% in the SD rat and about 30% in the GEPR.

The most dramatic increase in the number of GABAergic neurons occurs in the central portion of the rostrocaudal extent of the IC. In contrast, the increase in total number of cells is relatively uniform. These data indicate for the rostral and caudal poles of the IC that the increase in total number of cells exceeds the increase in GABAergic cells. It is possible that in these regions another transmitter system may be affected in addition to the GABAergic system.

The cnIII and the MSO did not display increased

numbers of neurons in the GEPR as compared to the SD. These data as well as qualitative findings from other brain regions suggest that the increase in neuronal density is not a whole brain phenomena but is possibly restricted to the IC. While this does not rule out the possibility that other brain regions may be similarly affected, it suggests that the change observed in the IC may be preferential.

#### *Conclusion*

These results indicate a change in the GABAergic system in the IC of the GEPR. Previous studies in this brain region suggest several abnormalities in its pharmacology and physiology<sup>2-5</sup> but this is the first report of an anatomical difference. Biochemical studies show an increase in the levels of glutamic acid decarboxylase (GAD) and GABA in the IC of the GEPR as compared to the control. Although these increases are not statistically significant, they were in the range of about 10% over normal levels<sup>6</sup>. These data support our results of an increase in the number of GAD-positive neurons in the GEPR.

Previous studies of these models have indicated deficits in two other neurotransmitters, norepinephrine and serotonin<sup>7-12,15-17</sup>, so it is unlikely that the GABAergic defect is the only problem. Since the norepinephrine system is one of the first neurotransmitter systems to develop, it may play a role in the generation of increased numbers of GABAergic neurons in the IC of the GEPR. The control of audiogenic seizures is very complex and undoubtedly involves an interplay of transmitters and possibly pathways. One question that remains unanswered is how these changes in the IC relate to motor seizures. A possible pathway is from the IC to the spinal cord either via the: (1) tectospinal tract or (2) reticulospinal projection. Evidence for the support of the latter is that lesions of the midbrain reticular formation<sup>15</sup> abolish audiogenic seizures in rats. Since the integrity of the IC is also necessary for the propagation of seizures it follows that some efferent projection that connects to the motor system may be an anatomical substrate for these seizures.

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